

MICROPIPETTING PITT KIT

Instructions for P1000, P200, and P20 Practice Exercise

In the biology laboratory we routinely use microliters (abbreviated μl) as a unit of measure for volumes. **One microliter = one-millionth of a liter = 10^{-6} liters = one-thousandth of a milliliter (ml).** This exercise tests your accuracy in setting up simulated reactions as you measure volumes from $2\mu\text{l}$ to $1000\mu\text{l}$ using P20, P200, and P1000 micropipettors. The success of your experiments depends on your skill in accurately measuring microliter volumes, so work carefully.

PART 1: SETTING UP REACTIONS IN THREE VOLUME RANGES

On your lab bench are a series of microtubes containing colored water, labeled **Practice Solution 1** through **Practice Solution 3**, and a jar of empty microtubes. **Each student** should use a permanent marker to label the *top* of three *empty* microtubes **Mock A**, **Mock B**, and **Mock X**. Use the table below as a checklist, marking off when you have added each practice solution to each mock reaction microtube. Remember to:

- **Choose the micropipettor whose volume range includes the volume you wish to measure.**
- **Select the appropriate colored tip for the micropipettor chosen.**
- **Hold your microtube at eye level** to see that your tip stays within the liquid you are measuring, and that an appropriate amount of liquid ends up in the tip with no air bubbles.
- **Dispense your liquid to the bottom of the microtube.** Don't squirt it into the top hoping the droplet falls in, and don't dispense the liquid to the side wall of the microtube where it might not mix with the other contents. If necessary, briefly centrifuge your microtube or tap its bottom on the bench to collect all its contents onto the bottom of the microtube.
- **Replace the micropipettor tip between the addition of every solution** to avoid cross-contamination of solutions.

	Practice Solution 1	Practice Solution 2	Practice Solution 3	
Tubes	Volumes of Practice Solutions to Add			Total Volumes
Mock A	50μl	125μl	25μl	200μl
Mock B	12μl	6μl	2μl	20μl
Mock X	350μl	275μl	375μl	1000μl

PART 2: CHECKING YOUR ACCURACY

Set a micropipetter to the TOTAL VOLUME for your **Mock A** microtube. Keeping the end of your tip near the bottom of the microtube, use this micropipetter to pick up all of the solution from your **Mock A** tube.

- Is the **tip full (no air bubble in the tip)** and is there **no liquid left in your microtube**? **Congratulations!** You have accurately pipetted the solutions to create a correct reaction.
- Is the **tip full (no air bubble in the tip)** but is there a **small volume of liquid left in the microtube**?
 - You may have incorrectly pushed the plunger to its second stop when measuring and picking up one of your practice solutions.
 - You may have added one of the solutions more than once.
 - Review your micropipetting technique with your instructor and try setting up this reaction again.
- After picking up all the liquid, is an **air bubble left at the end of the tip**?
 - You may have forgotten to add a solution.
 - When measuring a liquid, you may have let the plunger snap up too quickly. This can cause the liquid to splash within the tip and form drops that are not be dispensed into the reaction microtube. When measuring a liquid, release the plunger slowly and smoothly.
 - When measuring a liquid, you may have released the plunger without keeping the tip within the liquid. This often happens when the level of the liquid in the microtube falls as we remove the desired volume of liquid. Hold you microtube at eye level and watch that your tip remains in the liquid.
 - When dispensing a liquid, you may have released the plunger before removing it from the microtube and while the tip was still in contact with the liquid. This can cause some liquid to be sucked back into the tip.
 - Review your micropipetting technique with your instructor and try setting up this reaction again.

Repeat this accuracy test for your **Mock B** and **Mock X** microtubes.